Vol. VIII, No. 1, Suppl. 1 (March) 1975

In Vitro Reactivity of 3-M KCl-Solubilized Murine Histocompatibility (H-2) Antigens

A. Ahmed, K. W. Sell, R. C. Knudsen, D. M. Strong, and W. E. Vannier

IT IS WELL ESTABLISHED that when allogeneic cells are cultured together in vitro they give rise to stimulation and blast transformation (the MLC).[1,2] The mechanisms and genetic basis for this reaction have been a matter of intensive investigation. It was thought that the major histocompatibility HL-A antigens in man and H-2 antigens in the mouse were mainly responsible for this reactivity. However, recent evidence has demonstrated the existence of MLC reactivity between cells from mice belonging to the same H-2 allele.[3] These studies have led to the definition of the M locus[4,5] which is not linked to the major histocompatibility locus. In addition, MLC reactivity has also been demonstrated between mice differing at the thy. 1 marker,[6] and between mice with L^d differences.[7] Although humoral immunity has been associated with the major H-2 loci, a recent study has shown that non-H-2 antigens can generate a strong antibody response.[8] Our investigations were carried out to study the ability of solubilized extracts of mouse lymphoid cells to act as antigenic determinants of histocompatibility loci in various in vitro assays of cellular immunity.

MATERIALS AND METHODS

Mice. Mice belonging to the inbred strains BALB/c, C3H/He, CBA, DBA/2, AKR, and B10·D2 were obtained from the Jackson Laboratory, Bar Harbor, Me.

Antigen Extracts. Batches of spleens from an individual strain of mice were teased by means of a rubber policeman and H-2 antigens solubilized by 3 M KCl according to the method of Reisfeld et al.,[9] and further purified and quantitated by cytotoxicity inhibition assays using a panel of mouse alloantisera, as described previously.[10] Peak II of the G-200 column fractionated antigens[10] were used throughout these studies.

Media. RPMI 1640 containing 100 U/ml of penicillin and 100 μg/ml of streptomycin, 2 mM L-glutamine (Grand Island Biologicals, N.Y.), 25 mM Hepes and 10% heat inactivated fetal calf serum (Microbiological Associates, Bethesda, Md.) was used throughout as media.

Cell Cultures. The techniques for MLC, cell cultures and thymidine uptake measurements in a micro method have been published elsewhere.[11,12]

Migration Inhibition Assay. This assay was carried out by the indirect procedure as outlined by Ahmed et al.[13] Essentially, spleen cells 5×10^6/ml were cultured in a volume of 5 ml in sterile plastic test tubes (Falcon #3030, Oxnard, Calif.) for 48 hr in the presence of media or media containing varying dilutions of solubilized histocompatibility antigens. After the incubation period, the cultures were centrifuged at 1500 × g for 15 min at 4°C and the cell-free supernatant fluid was concentrated to a volume of 1 ml, either by dialysis or by Amicon UM-2 ultrafiltration. This fluid was then passed through a 0.22 μ Swinnex millipore filter and tested for MIF activity using guinea pig macrophages as the indicator cells.

Lymphotoxin Assay. The lymphotoxin assay was carried out by the method of Knudsen et al.[14]

Cell-Mediated Lymphotoxicity (CML) Assay. Groups of mice were either injected with 100 μg of solubilized antigen or 10×10^6 spleen cells either weekly, biweekly or three times a week for three weeks. Twenty-four hours after the last immunization, spleens were removed and spleen cells used as effector cells against either L-1210 or 129-P mouse ascites

From the Department of Clinical and Experimental Immunology, Naval Medical Research Institute, Bethesda, Md.

Supported by the Bureau of Medicine and Surgery, Department of the Navy, Research Task No. MF51.524.013.1004 A2BC. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The animals used in this study were handled in accordance with the provisions of Public Law 89-44 as amended by Public Law 91-579, the "Animal Welfare Act of 1970" and the principles outlined in the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

tumor cells in the ratio of 100:1. The L-1210 cells were carried in DBA/2 mice whereas the 129-P ascites cells were carried in C3H mice. The target cells were labelled with 300 μCi of ^{51}Cr ($Na^{51}CrO_4$, Amersham Searle, Boston, Mass.). Ten-thousand target cells were used for culture and 1×10^6 effector cells were added and the cultures incubated with loose caps in a rotator for 18 hr at 37°C in a 5% CO_2 humidified atmosphere. The cultures were performed in triplicates and after 18 hr of the culture period, the tubes were centrifuged and 100 μl of the supernatant fluid taken off and the amount of ^{51}Cr-release determined using a Nuclear Chicago gamma counter. The % Specific ^{51}Cr-release was calculated as: [(Average of experimental c.p.m.-Average spontaneous release)/(Average of 100% lysis-Average spontaneous release)] × 100.

RESULTS

Baseline studies were performed to determine the optimum concentration of solubilized antigens that gave maximum stimulation in the micro method. These studies showed that strains differed considerably in the amount of antigen required for optimal stimulation. Subsequently, these optimum concentrations of antigens were used with their respective strain combinations. Maximum stimulation occurred on day 4 with most combinations studied. As seen in Fig. 1, when spleen cells from DBA/2, B10·D2, BALB/c, C3H, CBA, AKR, C57BL/6 and C57BL/10 mice were incubated with DBA/2 solubilized antigen (optimum concentration), not only did it stimulate spleen cells from mice which differ at the H-2 allele, but it significantly stimulated BALB/c-spleen cells belonging to the same H-2^d allele. Similarly CBA solubilized antigens stimulated spleen cells from mice differing at the H-2 allele and also mice belonging to the same H-2^k allele (C3H and AKR). The degree of stimulation obtained with the solubilized extracts was similar whether from the same or a different H-2 allele. In efforts to investigate whether the antigen in the extracts was a T-cell product or a B-cell product, antigen was extracted from pools of thymus tissue or

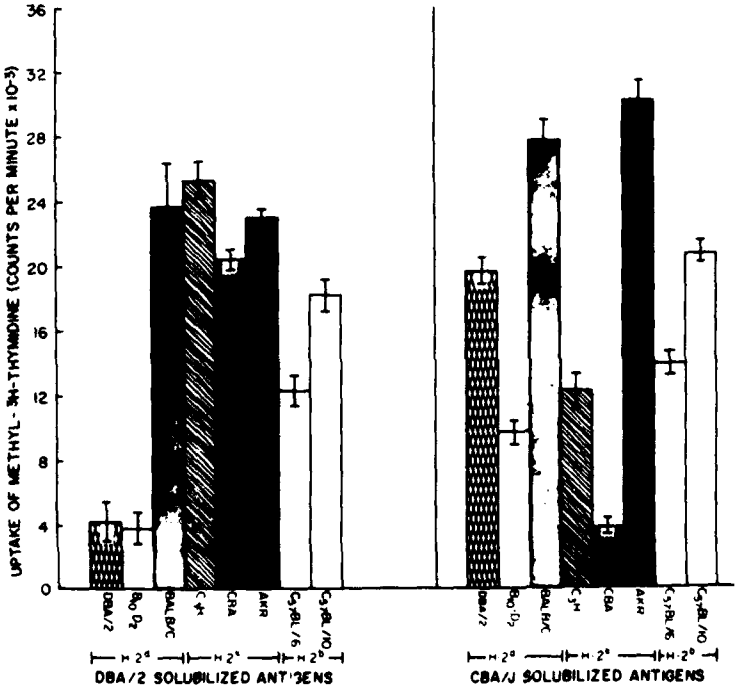


Fig. 1. In vitro stimulation of various strains of mice belonging to the H-2^d, H-2^k, and H-2^b allele with 3-M KCl-solubilized antigens from DBA/2 or CBA/J mice.

Table 1. In Vitro Stimulation of Thymocytes and Spleen Cells by 3-M KCl-solubilized DBA/2 and C3H Antigens

Stimulator 3M KCl Extract	Responder Cells	H-2/M Type	Uptake of ^{3}H-Thymidine (CPM ± S.E.) Mean of 3 Experiments
--	BALB/c spleen	$^{d}/_{M}{}^{2}$	2129 ± 107
--	BALB/c thymus	$^{d}/_{M}{}^{2}$	413 ± 36
--	DBA/2 spleen	$^{d}/_{M}{}^{1}$	3734 ± 154
--	DBA/2 thymus	$^{d}/_{M}{}^{1}$	189 ± 41
--	C3H/He spleen	$^{k}/_{M}{}^{3}$	5996 ± 484
--	C3H/He thymus	$^{k}/_{M}{}^{3}$	244 ± 33
DBA/2 thymus	BALB/c spleen	$^{d}/_{M}{}^{2}$	1889 ± 90
DBA/2 thymus	BALB/c thymus	$^{d}/_{M}{}^{2}$	137 ± 27
DBA/2 thymus	DBA/2 spleen	$^{d}/_{M}{}^{1}$	2250 ± 136
DBA/2 thymus	DBA/2 thymus	$^{d}/_{M}{}^{1}$	237 ± 28
DBA/2 thymus	C3H/He spleen	$^{k}/_{M}{}^{3}$	12369 ± 647
DBA/2 thymus	C3H/He thymus	$^{k}/_{M}{}^{3}$	4871 ± 293
DBA/2 spleen	BALB/c spleen	$^{d}/_{M}{}^{2}$	26351 ± 1895
DBA/2 spleen	BALB/c thymus	$^{d}/_{M}{}^{2}$	7119 ± 766
DBA/2 spleen	DBA/2 spleen	$^{d}/_{M}{}^{1}$	3275 ± 618
DBA/2 spleen	DBA/2 thymus	$^{d}/_{M}{}^{1}$	486 ± 55
DBA/2 spleen	C3H/He spleen	$^{k}/_{M}{}^{3}$	29183 ± 3210
DBA/2 spleen	C3H/He thymus	$^{k}/_{M}{}^{3}$	3774 ± 406
BALB/c spleen	DBA/2 spleen	$^{d}/_{M}{}^{1}$	3984 ± 345
BALB/c spleen	DBA/2 thymus	$^{d}/_{M}{}^{1}$	516 ± 67
BALB/c spleen	BALB/c spleen	$^{d}/_{M}{}^{2}$	2551 ± 287
BALB/c spleen	BALB/c thymus	$^{d}/_{M}{}^{2}$	183 ± 53
BALB/c spleen	C3H/He spleen	$^{k}/_{M}{}^{3}$	18958 ± 2125
BALB/c spleen	C3H/He thymus	$^{k}/_{M}{}^{3}$	6484 ± 713

spleen cells. As seen in Table 1, antigen extracts from DBA/2 thymus cells did not stimulate BALB/c spleen or thymus cells, but did stimulate C3H/He spleen and thymus cells. On the other hand, antigen extracts from DBA/2 spleen cells stimulated both BALB/c and C3H/He spleen and thymus cells. Conversely, extracts from BALB/c spleen cells did not stimulate DBA/2 spleen or thymus cells but did stimulate spleen and thymus cells from C3H/He mice. These data suggest that the stimulatory antigen present in the DBA/2 extract is found in cells other than T cells. Similar results were obtained which showed that only CBA spleen antigen extracts, but not CBA thymus cell extracts, stimulated C3H thymus, and spleen cells. To determine whether the peripheral T-cells also lacked this antigen, experiments were carried out

where DBA/2 spleen cells were used as stimulators after treatment with anti-θ C3H serum and complement. These cells were put on a Ficoll-hypaque gradient (sp. gr. 1.094) to remove dead cells. Results showed that BALB/c spleen cells responded to anti-θ treated DBA/2 spleen cells equally as well as untreated DBA/2 spleen cells (Table 2). Reactivity to nonspecific T-cell mitogens, PHA and Con-A, were used as an index of the removal of T cells. Results from this experiment also show that T cells are necessary in the responder population. When adherent cells were removed from DBA/2 spleen cells after anti-θ treatment, they still stimulated BALB/c spleen cells though they showed slightly lower reactivity (9436 ± 401 as compared to 22147 ± 1105). When CBA spleen cells were used to stimulate C3H, similar results were obtained.

To determine if these DBA/2 extracts could induce sensitization in vivo, mice belonging to the same H-2^d allele, BALB/c, and B10·D2, and mice belonging to the H-2^k allele, C3H/He, AKR, and CBA, were injected with varying doses of the solubilized extract subcutaneously or intraperitoneally with varying schedules from one a week to three times a week for a period of three weeks. For comparison, mice were injected with 10×10^6 whole spleen cells. As seen in Table 3, solubilized extracts failed to induce cytotoxic effector cells. On the other hand, whole spleen cells induced specific cytotoxic effector cells in mice differing at the H-2 allele but little or no effect

Table 2. Role of DBA/2 Normal T and B Cells in Stimulating BALB/c and C3H Spleen Cells*

Stimulator Cells† and Treatment	Responder Cells and Treatment	Uptake of methyl-^{3}H-thymidine (Counts per minute ± S.E.)
DBA/2	---	193 ± 34
--	DBA/2	2339 ± 210
BALB/c	---	311 ± 46
--	BALB/c	3896 ± 248
DBA/2	BALB/c	26241 ± 1593
BALB/c	DBA/2	4415 ± 238
DBA/2 (aθ+C')	BALB/c	22147 ± 1105
DBA/2	BALB/c (aθ+C')	3126 ± 150
DBA/2 (aθ+C')	BALB/c (aθ+C')	1432 ± 94
DBA/2	C3H/He	18359 ± 844
DBA/2 (aθ+C')	C3H/He	12406 ± 533
DBA/2	C3H/He (aθ+C')	3012 ± 141
DBA/2 (aθ+C')	C3H/He (aθ+C')	1299 ± 234
DBA/2 (aθ+C') nonadherent	BALB/c	9436 ± 401

*7×10^5 responder cells were cultured with 8×10^5 stimulator cells for 96 hours in a microculture system.

† Stimulator cells were treated with 25 µg/ml mitomycin-C.

Table 3. CML Reactivity of Allogeneic Sensitized Spleen Cells

Sensitized with	Responder and Effector Cells	H-2 Type	% Specific Net ^{51}Cr Release: L-1210 Target Cell ($H\text{-}2^d$)	% Specific Net ^{51}Cr Release: 129-P Target Cell ($H\text{-}2^k$)
DBA/2 Extract	DBA/2		2.9	0.8
	BALB/c	$H\text{-}2^d$	3.7	-3.1
	B10·D2		-3.6	2.7
	C3H/He		-1.2	6.1
	AKR	$H\text{-}2^k$	2.7	4.3
	CBA/J		6.9	2.7
DBA/2 Cells	DBA/2		3.3	2.9
	BALB/c	$H\text{-}2^d$	0.6	3.7
	B10·D2		1.9	0.5
	C3H/He		27.3	1.3
	AKR	$H\text{-}2^k$	18.7	0.3
	CBA/J		23.8	-3.0

in BALB/c or B10·D2 which belong to the same $H\text{-}2^d$ allele. Similar results were obtained with CBA/J solubilized antigens. Mice injected with solubilized extracts, however, did produce antibodies in allogenic combinations as tested by cytotoxicity.

In order to determine if these in vitro and in vivo responses can be enhanced by prior sensitization, experiments were carried out where C3H/He, C57BL/6 and BALB/c mice were immunized with DBA/2 spleen cells and subsequently skin grafted. Syngeneic mice were used as controls. After complete rejection of skin grafts, spleen cells were removed and cultured with DBA/2 solubilized extracts. As seen in Fig 2, C3H mice sensitized to DBA/2 (designated as C3H, DBA/2) gave twice as many counts as nonsensitized (see Fig. 1). Similar results were seen with BALB/c, DBA/2 and C57BL/6, DBA/2. Peak stimulation occurred on day 3, and not on day 4. When similar sensitized mice were studied after different days post skin transplantation, it was of interest that in nearly all strains tested there was a marked depression between days 4–6 in both whole spleen cell MLC and cell stimulation by the use of solubilized extracts from the sensitizing strain (Fig. 3). By day 10–12, there was a hyperstimulation which was about twice that observed with nonsensitized controls. In another experiment, varying concentrations of cells from day 5 BALB/c sensitized spleen cells were added to day 12 BALB/c sensitized spleen cells, which were then used in MLC with mitomycin-C treated DBA/2 spleen cells. As seen in Table 4, increasing concentrations of day 5 spleen cells inhibited the response of day 12 spleen cells. The maximum effect occurred with as little as 3×10^5 day 5 spleen cells when added to 4×10^5 day 12 spleen cells. To determine if this inhibitory effect was due to T-cell function or B-cell function, 3×10^5 anti-θ + C′ treated day 5 spleen cells were added to day

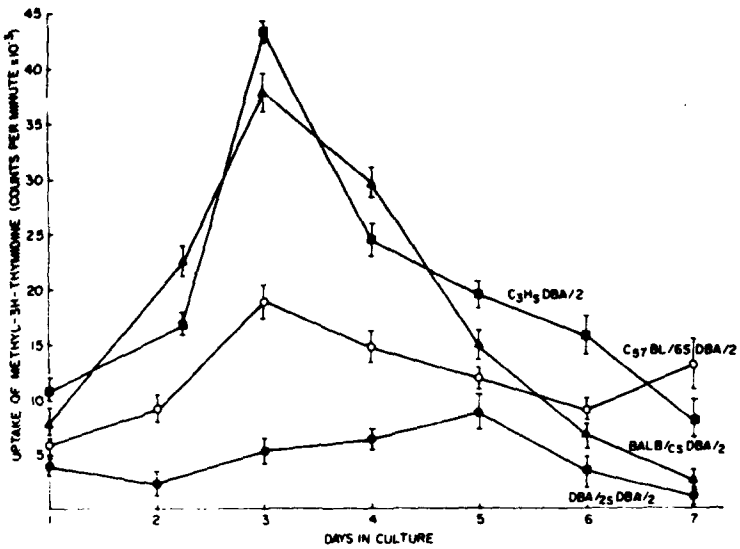


Fig. 2. Kinetics of the in vitro stimulatory response of spleen cells from C3H, C57BL/6, BALB/c, and DBA/2 sensitized to skin allografts from DBA/2 mice (designated $C3H_s$ DBA/2, etc.) in the presence of 3 M KCl-solubilized DBA/2 antigens.

12 spleen cells. It is seen that the inhibitory effect is not removed by such treatment.

When supernatant fluids from cultures of normal or sensitized cells incubated with solubilized antigens were assayed for levels of lymphotoxin (LT), it was seen that normal BALB/c spleen cells produced just as much LT as C3H/He spleen cells when incubated with DBA/2 antigen (Table 5). Conversely, DBA/2 spleen cells produced a low level of LT when incubated with BALB/c antigens. When sensitized cells were used in such an assay system, it was found that the maximum level of LT was produced by spleen cells from animals 5 days post skin graft sensitization with both $C3H_sDBA/2$ (which increased from 1:26.8 to $\geq$ 1:320) or $BALB/c_s$ DBA/s (which increased from 1:37.5 $\geq$ 1:360). Conversely, BALB/c antigen induced very low levels of

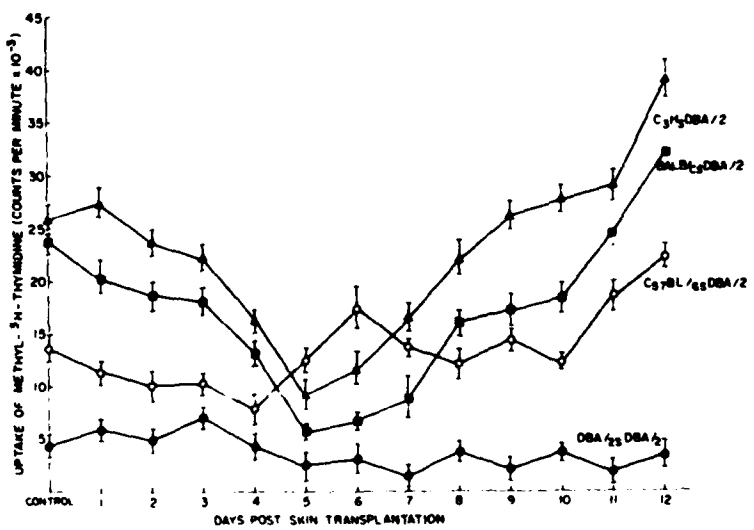


Fig. 3. In vitro response of C3H, BALB/c, C57BL/6, and DBA/2 mouse spleen cells to 3 M KCl-solubilized DBA/2 antigens during varying days postskin transplantation from DBA/2 mice.

Table 4. Effect of Inhibitor (Day-5-sensitized) BALB/c Responding Cells on Hyperactive (Day-12-sensitized) BALB/c Responding Cells in MLC with DBA/2 Spleen Cells*

No. of 12 Day Sensitized Cells	No. of 5 Day Sensitized Cells	Uptake of methyl-^{3}H-thymidine (Counts per minute ± S.E.)
7×10^5	--	36189 ± 1554
6.5×10^5	0.5×10^5	43274 ± 2998
6×10^5	1×10^5	30556 ± 1931
5×10^5	2×10^5	21846 ± 1448
4×10^5	3×10^5	7933 ± 612
3×10^5	4×10^5	4895 ± 24
2×10^5	5×10^5	5163 ± 298
1×10^5	6×10^5	4118 ± 317
0.5×10^5	6.5×10^5	5637 ± 389
---	7×10^5	4813 ± 512
4×10^5	3×10^5 ($\alpha\theta$+C')	7137 ± 344

*8×10^5 DBA/2 spleen cells/culture which were pretreated with 25 μg/ml mitomycin-C were used as stimulator cells and the cultures harvested after 96 hours of incubation.

LT when incubated with normal DBA/2 spleen cells or spleen cells from DBA/2 mice obtained on different days after skin transplantation from BALB/c mice.

Lastly, when supernatant fluids of spleen cells incubated with solubilized antigens were assayed for MIF activity, it was found that normal spleen cells did not produce any MIF activity. BALB/c or C3H mice were sensitized by injection with 10×10^6 whole DBA/2 spleen cells or with 100 μg of solubilized DBA/2 antigens three times a week for three weeks and subsequently challenged with skin grafts from DBA/2 mice. Their spleen cells were removed on different days post-transplantation and incubated with varying concentrations of DBA/2 solubilized extracts for 48 hr. The supernatant fluids were then assayed for MIF activity and it was found that significant MIF activity could be detected as early as day 5 post-transplantation in these sensitized spleen cell cultures (Fig. 4).

DISCUSSION

Biological activity in terms of induction of transformation of allogeneic cells by solubilized extracts has been shown by several workers.[2,15-17] Some investigations[18-20] have shown that normal lymphocytes show less in vitro stimulation as compared to sensitized lymphocytes. Young and Gyenes[21] were able to demonstrate cytotoxic effector cells in mice immunized with freeze-thaw extracts of allogeneic lymphoid cells. On the other hand, Zighelboim et al.,[22] using soluble antigen extracts from EL-4 cells, demonstrated blocking activity for cell-mediated lympholysis. Most studies[23] show that pretreatment of recipients with solubilized allogeneic histocompatibility antigens induce accelerated skin graft rejection

Table 5. Level of Lymphotoxin (ID_{50}) in Spleen Cells from Mice (Normal and Allograft-sensitized) in Response to 3-M KCl-solubilized Histocompatability Antigens

Antigen	Target Spleen Cells		Level of Lymphotoxin (ID_{50})
DBA/2	Normal BALB/c		1:37.5
DBA/2	Normal C3H		1:26.8
DBA/2	Normal DBA/2		< 1:2
DBA/2	$C3H_S$ DBA/2	day 1	1:32
DBA/2	$C3H_S$ DBA/2	day 3	1:68
DBA/2	$C3H_S$ DBA/2	day 5	> 1:320
DBA/2	$C3H_S$ DBA/2	day 7	1:180
DBA/2	$C3H_S$ DBA/2	day 10	1:196
DBA/2	$C3H_S$ DBA/2	day 14	1:128
DBA/2	$C3H_S$ DBA/2	day 21	1:64
DBA/2	$BALB/c_S$ DBA/2	day 1	1:96
DBA/2	$BALB/c_S$ DBA/2	day 3	1:100
DBA/2	$BALB/c_S$ DBA/2	day 5	> 1:360
DBA/2	$BALB/c_S$ DBA/2	day 7	> 1:360
DBA/2	$BALB/c_S$ DBA/2	day 10	1:300
DBA/2	$BALB/c_S$ DBA/2	day 14	1:256
DBA/2	$BALB/c_S$ DBA/2	day 21	> 1:100
BALB/c	Normal DBA/2		1:4
BALB/c	$DBA/2_S$ BALB/c	day 1,3, 5,7,10,14,21	between < 1:2 to 1:10

whereas some studies show prolongation[24] or enhancement of rat kidney allografts.[25]

In the present studies, we have demonstrated the isolation of solubilized antigens that induce blastogenesis in not only spleen cells from mice that differ at the H-2 allele but also mice that differ at the M locus as described by Festenstein.[4,5] These antigens appear to be present on nonthymic derived cells and appear not to be capable of eliciting cytotoxic effector cells but do induce the production of lymphotoxin in both unsensitized and sensitized lymphoid cells. However, only sensitized cells demonstrated MIF production upon incubation with the sensitizing allogeneic solubilized antigen extracts.

When spleen cells from mice following skin transplantation are used as responders in the presence of the sensitizing antigen extracts or whole spleen cells, a marked depression in the degree of stimulation was seen on day 5. There is, however, increased stimulation by day 12. Inhibition of the MLC response following transplantation has been previously reported by Miller et al.[26] The cells responsible for this inhibitory effect in our system seem to be cells other

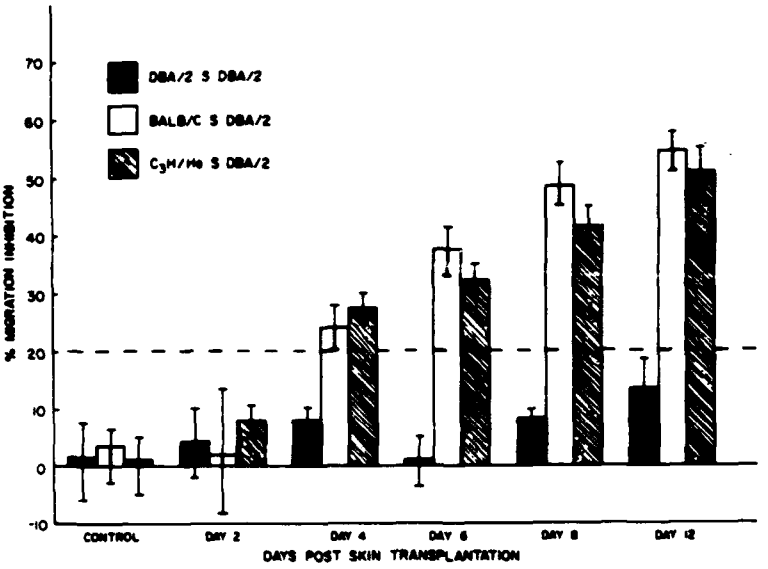


Fig. 4. Production of migration inhibitory factor by spleen cells from DBA/2, BALB/c, and C3H mice in response to 3 M KCl-solubilized DBA/2 antigens on various days after skin transplantation from DBA/2 mice.

than T cells or adherent cells. Interestingly, the time of occurrence of high levels of LT corresponds to the day where there is marked decrease in stimulation with solubilized extracts. This is also about the time where MIF production is induced. The mechanisms of the interaction of these factors and the various interpretations of these findings could be better studied with the use of congenic strains of mice which differ at the M locus.

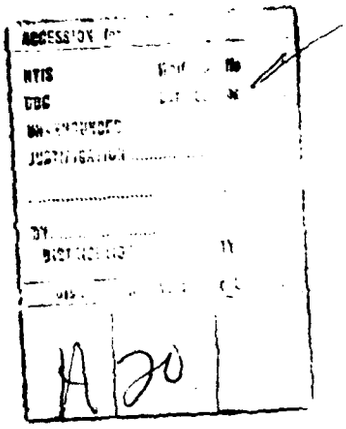
ACKNOWLEDGMENTS

The authors are deeply grateful to Dr. I. Scher for his helpful comments and criticisms, and acknowledge the technical assistance of HN Dayton Naugle, HM2 Martin Van Cleave; Timothy E. Brown and Betty J. Sylvester for typing this manuscript.

REFERENCES

1. Bain B, Vas MR, Lowenstein L: Blood 23:108, 1964
2. Oppenheim JJ: In: Kahan BD, Reisfeld RA (eds), Transplantation Antigens, New York, Academic Press, 1972, p 357
3. Peck AB, Click RE: Transplantation 16:331, 1973
4. Festenstein H: Ann NY Acad Sci 129:567, 1966
5. Festenstein H, Abbasi K, Sachs JA, et al: Transplant Proc IV(2):219, 1972
6. Peck AB, Click RE: Transplantation 16(4):339, 1973
7. Bach FH, Widmer MB, Bach ML, et al: J Exp Med 136:1430, 1972
8. Baldwin WM, Cohen N: Immunogenet 1:33, 1974
9. Reisfeld RA, Pellegrino MA, Kahan BD: Science 172:1134, 1972
10. Ahmed A, Thurman GB, Vannier WE, et al: J Immunol Meth 3(1):1, 1973
11. Strong DM, Ahmed A, Thurman GB, et al: J Immunol Meth 2:279, 1973
12. Thurman GB, Strong DM, Ahmed A, et al: Clin Exp Immunol 15:289, 1973
13. Ahmed A, Strong DM, Thurman GB, et al: J Exp Med 139:902, 1974
14. Knudsen RC, Ahmed A, Sell KW: J Immunol Meth 5:55, 1974
15. Ranney DF, Gordon RO, Pincus JH, et al: Transplantation 16(6):558, 1973
16. Viza DC, Degani O, Dausset J, et al: Nature 219:704, 1968
17. Manson LA, Simmons T: Transplant Proc I (1):498, 1969
18. Gutterman JU, Mavligit G, McCredie KB, et al: Science 177:1114, 1972
19. Leventhal BG, Mann DL, Rogentine GN: Transplant Proc III(1):243, 1971

20. Austoker JL, Little J, Myburgh JA: S Afr J Surg 11(3):128, 1973
21. Young JM, Gyenes L: Cell Immunol 6:231, 1973
22. Zighelboim J, Bonavida B, Rao VS, et al: J Immunol 112(1):433, 1974
23. Graff RJ, Mann DL, Nathenson SG: Transplantation 10:59, 1970
24. Kilshaw PJ, Brent L, Thomas AV: Transplantation 17(1):57, 1974
25. Von Haefen U, Shaipanich T, Wren S, et al: Transplant Proc V(1):573, 1973
26. Miller J, Hattler BG Jr, Johnson MC: Transplantation 14(1):57, 1972



UNCLASSIFIED

DOCUMENT CONTROL DATA - R & D

NAVAL MEDICAL RESEARCH INSTITUTE
BETHESDA, MARYLAND 20014

UNCLASSIFIED

IN VITRO REACTIVITY OF 3-M KCl-SOLUBILIZED MURINE HISTOCOMPATIBILITY (H-2) ANTIGENS.

MEDICAL RESEARCH PROGRESS REPORT.

AHMED, [redacted] SELL, [redacted] KNUDSEN, [redacted] STRONG [redacted] VANNIER [redacted]

REPORT DATE	TOTAL NO OF PAGES	NO OF REFS
1975	10	23

PROJECT NO 16. MF51-524

ORIGINATOR'S REPORT NUMBER(S) 17) MF51-524-013, ~~1004 Report No.~~ 1

OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

11) 1974

DISTRIBUTION STATEMENT

THIS DOCUMENT HAS BEEN APPROVED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED

SUPPLEMENTARY NOTES

Trans. Proc., 3(1):213-222, 1975

SPONSORING MILITARY ACTIVITY

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND, NNMC, BETHESDA, MARYLAND 20014

12) 11p.

ABSTRACT

It is well established that when allogeneic cells are cultured together in vitro they give rise to stimulation and blast transformation (the MLC).[1,2] The mechanisms and genetic basis for this reaction have been a matter of intensive investigation. It was thought that the major histocompatibility HL-A antigens in man and H-2 antigens in the mouse were mainly responsible for this reactivity. However, recent evidence has demonstrated the existence of MLC reactivity between cells from mice belonging to the same H-2 allele.[3] These studies have led to the definition of the M locus[4,5] which is not linked to the major histocompatibility locus. In addition, MLC reactivity has also been demonstrated between mice differing at the thy. 1 marker,[6] and between mice with Ld differences.[7] Although humoral immunity has been associated with the major H-2 loci, a recent study has shown that non H-2 antigen can generate strong antibody response.[8] Our investigations were carried out to study the ability of solubilized extracts of mouse lymphoid cells to act as antigenic determinants of histocompatibility low in various in vitro assays of cellular immunity.

DD FORM 1473

UNCLASSIFIED

KEY WORDS

Antigens
Cells (Biology)
Cultures (Biology)
Mice
Assaying